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Dr. Ed Southern
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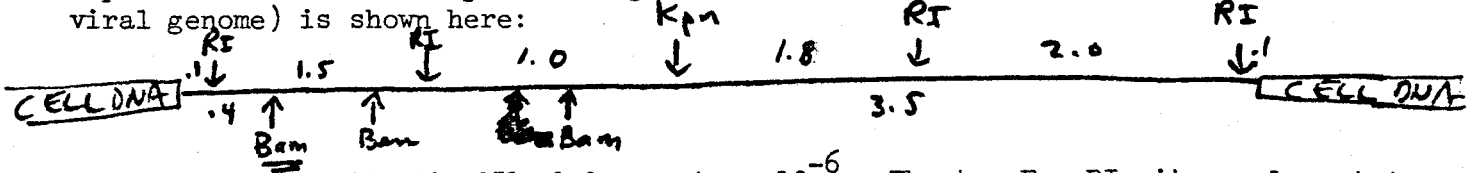
Dear Ed:

We are writing to enlarge our previous discussions about cloning integration sites for the DNA of avian sarcoma viruses. We would like your opinion on the scientific and political merits of the various options.

It would be easiest to begin with DNA from mammalian cells cloned after infection with ASV. Mammalian cells are much easier to grow than avian cells (chicken cells transformed with ASV are difficult to clone and are not immortal in culture); they have the additional advantage of being non-permissive for viral replication, although most or all of the viral genes are expressed at a low level. We have a series of rat cell (NRK) clones infected with and transformed by the Schmidt Ruppin D strain of ASV. Several of these clones contain a single copy of integrated DNA and we have identified restriction fragments which contain viral DNA linked to cell DNA. The RNA genome of the virus is diagramatically represented below:

It is roughly 10,000 bases long and contains at least (and probably not much more than) four genes. Gag is the gene coding for a polyprotein which is processed into several core components. Pol is the reverse transcriptase gene. Env is the major envelope glycoprotein gene, and src is a gene, dispensable for viral replication, which causes the transformation of fibroblasts in culture and the formation of sarcomas in vivo.

A partial restriction map for integrated viral DNA (drawn to be co-linear with the viral genome) is shown here:



The numbers refer to the MW of fragments x 10^{-6}. The two Eco RI sites relevant to this discussion are very close to the ends of the DNA copy of the viral genome. We have used these sites to demonstrate that even in the non-permissive cells viral DNA usually integrates, probably via a circular intermediate, so that the gene is co-linear, or very nearly co-linear, with the RNA genome. The "5'" RI site is ~200bp from the "end" of the provirus and the "3'" RI site is about 60bp from the end which

corresponds to the 3' end of the RNA. We are particularly interested in the cellular sequences which are adjacent to the "5'" end of the provirus because the transcriptional control elements are likely to be located at that end of the genome.

We could propose to clone either the Eco RI or the BAM H_I fragments containing the "5'" portion of the provirus linked to cellular DNA. We would much rather clone the BAM fragment for two reasons. (i) It is easier to detect this fragment by hybridization since it has a larger region of homology with our standard reagents. (ii) More importantly, there are interesting sequences which lie between the RI and BAM sites. We have found, by hybridization with reagents specific for the terminal sequences, considerable homology between the "internal" region of the viral DNA and sequences from the 3' and 5' ends of the viral genome. We are therefore very interested in this segment. This region is likely to be important in the processing of mRNAs (as you may know by now, 100-500 bases from the 5' terminus are transposed or spliced onto the 5' ends of the subgenomic messages which code for env and src). In addition, this region might also contain the as yet unmapped amino terminus of the gag gene. We would however prefer to clone the Eco RI fragment than be refused permission to clone the BAM fragment. On could argue that it is safer to clone the RI fragment (~200bp of viral information) than the BAM piece (~600bp of viral information).
We would like to clone the "5'" fragments from at least three independent clones to obtain adequate comparative data. We now have mapping data on more than 10 clones which have a non-defective provirus integrated in different sites on the host genome. We can easily prepare in excess of 10 mg of DNA from each of the clones and can obtain sufficient enzyme to cleave this amount of material. We should be able to enrich the appropriate segment(s) of DNA by sequential enzymatic digestions interspersed with physical fractionations. We would propose to begin with a KPN digestion followed by a size separation using the "gene machine". The appropriate fragment can be located by a small scale DNA transfer experiment on the fractions from the gene machine. We can distinguish the "3'" and "5'" ends of the provirus with specific probes. The DNA could be further purified, if necessary, using an RPC-5 column. The DNA could then be digested with either BAM or RI and again fractionated by size. This should give us a preparation which is at least 10^3-10^4-fold enriched for the relevant fragment.

Fragments prepared by this protocol should be completely free of the viral src gene which is located in fragments of different size, derived from the other end of the provirus. This can be directly tested with cDNA specific for src.

If you forsee political problems caused by using DNA from a cell infected with a transforming (src +) virus, we could probably obtain an appropriate set of non-transformed clones infected with a deletion mutant of ASV which lacks most of src. The advantage of the transformed clones is that we already have a great deal of information about the maps of integrated provirus in these lines. If you think we should propose to use clones infected with a transformation-defective deletion mutant, it would be at least several months before we could have the DNA from the appropriate clones sufficiently well mapped to make specific proposals. We do not see any real advantage to the use of mutant-infected cells, since all vertebrate cells clearly contain multiple sequences related to various viral transforming genes. The ASV src gene can be readily measured and therefore excluded, whereas the other endogenous sequences have probably been unwittingly cloned many times already. (Needless to say, we do not believe such manipulations pose any threat to the body politic.)

Since we can choose to work with infected cells which yield "5'" BAM or RI fragments that are relatively large it should be possible to use one of the λ packaging systems. The λ system would be easier to use with RI than BAM. The obvious alternatives are pBR322 x 1776 and the yet to be unveiled Brenner strain.

Please let us know what you think about our proposals from both a scientific and a political point of view. We are looking forward to hearing from you.

Best regards,

Harold

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HEV/es